IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Appln. No.	: 10/527,093
Applicant	: You Sup CHUNG et al.
Filed	: March 9, 2005
TC/A.U.	: 1625
Examiner	: Patricia L. Morris
Docket No.	: 1751-376
Customer No.	: 06449
Confirmation No.	: 2748

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DECLARATION UNDER 37 C.F.R. '1.132

I, Munchoun Ha, declare as follows:

1. I am a co-inventor of the above-identified application.

2. I am currently employed in the Organic Synthesis Research Team at the Hanlim Pharmaceutical Central Research Institute.

3. I make this declaration in order to make of record the results of certain tests carried out in accordance with the present application.

4. The following experiments were conducted by me or under my supervision and control.

5. Preparation of test material

(1) Amlodipine nicotinate was prepared in accordance with Example 1 of US 6,699,892. However, according to a preliminary test result, the yield described in Example 1 of US 6,699,892 could not be obtained. Yields of 62.1% and 83.5%,

respectively, were obtained by standing for 4 hours and 8 hours, respectively, prior to filtering after 1 hour of cooling. Therefore, an amlodipine nicotinate was prepared by incorporating a step of standing for 8 hours prior to filtering after 1 hour of cooling (underlined), in the process described in Example 1 of US 6,699,892, as follows:

Amlodipine free base (4.09g) was added to 40 ml of ethanol. The mixture was heated at reflux and stirred until the solid was completely dissolved. Nicotinic acid (1.23g) was added to the amlodipine solution and then the mixture was slowly cooled down to 0℃ within one hour. The reaction mixture was maintained for 8 hours. The solids formed were isolated by filtration, washed with ethyl acetate and dried under reduced pressure to yield 4.440g of amlodipine nicotinate (Yield: 83.5%).

(2) An S-(-)-amlodipine nicotinate was prepared in accordance with Example 1 of US 6,699,892, using S-(-)-amlodipine instead of amlodipine. However, according to a preliminary test result, a yield described in Example 1 could not be obtained. Yields of 57.3% and 78.7%, respectively, were obtained by standing for 4 hours and 8 hours, respectively, prior to filtering after 1 hour of cooling. Therefore, an S-(-)-amlodipine nicotinate was prepared by incorporating a step of standing for 8 hours prior to filtering after 1 hour of cooling (underlined) and using S-(-)-amlodipine instead of amlodipine, in the process described in Example 1 of US 6,699,892, as follows:

S-(-)-amlodipine free base (4.09g) was added to 40 ml of ethanol. The mixture was heated at reflux and stirred until the solid was completely dissolved. Nicotinic acid (1.23g) was added to the S-(-)-amlodipine solution and then the mixture was slowly cooled down to 0°C within one hour. The reaction mixture was maintained for 8 hours. The solids formed were isolated by filtration, washed with ethyl acetate and dried under

reduced pressure to yield 4.183g of S-(-)-amlodipine nicotinate (Yield: 78.7%).

6. Measurement of water content

Water content of the amlodipine nicotinate and S-(-)-amlodipine nicotinate prepared in 5 was measured using a Karl Fisher device, and the results are shown in Table 1.

Table 1.

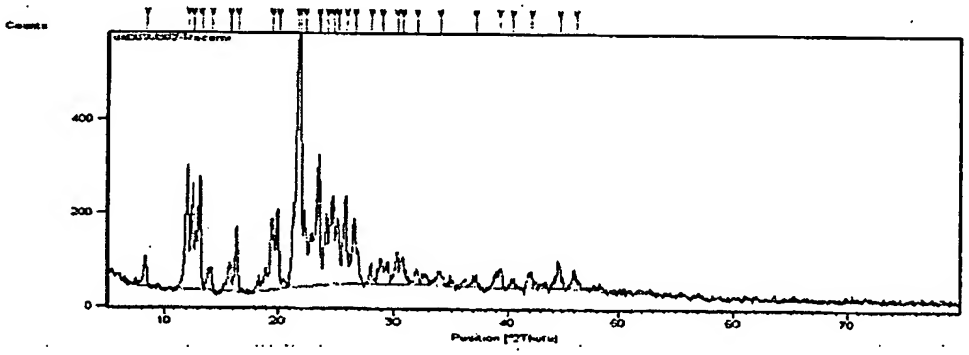
	Water content (%)
amlodipine nicotinate prepared in 5(1)	0.22
S-(-)-amlodipine nicotinate prepared in 5(2)	0.17

As shown in Table 1, the amlodipine nicotinate and S-(-)-amlodipine nicotinate prepared in 5(1) and 5(2) are presumed to be in an anhydrate form since the water content thereof is less than 0.5%.

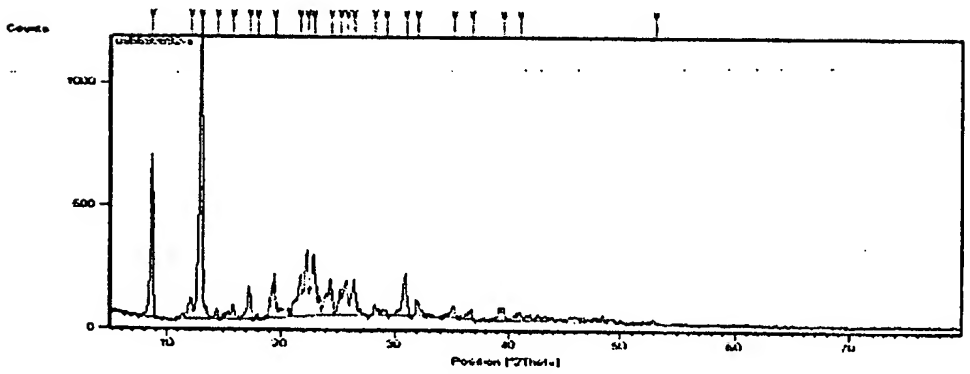
7. Measurement of the XRD pattern

XRD patterns of the amlodipine nicotinate and S-(-)-amlodipine nicotinate prepared in 5(1) and 5(2) were measured using an X-ray Diffractometer (PANalytical, X'pert-Pro), and the measurement results are as follows.

(1) XRD of amlodipine nicotinate prepared in 5(1)



(2) XRD of S-(-)-amlodipine nicotinate prepared in 5(2)



As shown in XRD patterns (1) and (2), the amlodipine nicotinate and S-(-)-amlodipine nicotinate prepared in 5(1) and 5(2) are presumed to be a different material from the S-(-)-amlodipine nicotinate in a dihydrate form of the present invention since they show a different XRD pattern from that shown in FIG 1. of the present application.

8. Measurement of solubility

Water solubility of the amlodipine nicotinate and S-(-)-amlodipine nicotinate prepared in 5(1) and 5(2) were measured. Further, water solubility of S-(-)-amlodipine nicotinate in a dihydrate form prepared in accordance with Example 1 of the present application was also measured.

More than 1g of each sample was added to 100ml of distilled water and saturated by ultrasonic extraction for 30 minutes. The saturated solution was filtered, and 5ml of permeate was carefully taken and added to a 100ml volume flask, and then water was added to fill the flask (20 fold dilution). Next, 1ml of the 20 fold diluted solution was carefully taken and added to a 50ml volume flask and then water was added to fill the flask (50 fold dilution) (1000 fold dilution in total). The 1000 fold diluted solution was used

as a test sample in spectroscopic analysis at 237nm.

In addition, a standard curve was prepared from each sample, 10.0mg of each sample was carefully taken and dissolved in distilled water and the final volume was set to 100ml by adding distilled water thereinto. 10ml of the resultant solution was added to a 50ml volume flask and then water was added to fill the flask. The standard solution was analyzed in triplicate by UV spectroscopy to prepare a standard curve. The results of the standard curve of each sample are shown in Table 2. The results of absorbance measurement of each sample are shown in Table 3.

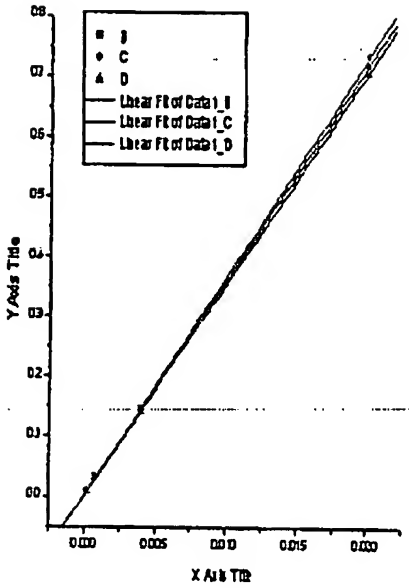
Table 2. Standard curve

Concentration (mg/ml)	B line	C line	D line
0.02	0.7182	0.7318	0.7075
0.004	0.1467	0.1468	0.1405
0.0008	0.0322	0.0306	0.0321
0.00016	0.0074	0.0089	0.0082

B line: Absorbance of amlodipine nicotinate prepared in 5(1)

C line: Absorbance of S-(-)-amlodipine nicotinate prepared in 5(2)

D line: Absorbance of S-(-)-amlodipine nicotinate in a dihydrate form prepared in accordance with Example 1 of the present invention.



[2006-11-22 02:13 *:Graph1* (245-1061)]
Linear Regression for Data1_B:
Y = A + B * X

Parameter	Value	Error
A	0.0029	7.03B-5E-4
B	35.77279	0.05905

R	SD	N	P
1	0.00712	4	<0.0001

[2006-11-22 02:13 *:Graph1* (245-1061)]
Linear Regression for Data1_C:
Y = A + B * X

Parameter	Value	Error
A	0.00184	7.3145E-4
B	36.48556	0.06196

R	SD	N	P
1	0.00716	4	<0.0001

[2006-11-22 02:13 *:Graph1* (245-1061)]
Linear Regression for Data1_D:
Y = A + B * X

Parameter	Value	Error
A	0.00215	0.00144
B	35.26517	0.14093

R	SD	N	P
0.99998	0.00228	4	<0.0001

Table 3. Absorbance of each sample

Repeat	B line	C line	D line
1	0.2251	0.2498	0.2651
2	0.2247	0.2501	0.2654
3	0.2251	0.2500	0.2647
Average (Y value)	0.2250	0.2500	0.2651
Standard curve	Y=35.77279X+0.0029	Y=36.48756X+0.00184	Y=35.2451X+0.00215
Dilution ratio	1000 fold	1000 fold	1000 fold
Calculated concentration	6.21mg/ml	6.80mg/ml	7.46mg/ml

As shown in Table 3, S-(-)-amlodipine nicotinate in a dihydrate form prepared in accordance with Example 1 of the present invention having a specific XRD pattern has an enhanced water solubility by about 20% compared to amlodipine nicotinate, and about 9.7% compared to S-(-)-amlodipine nicotinate.

9. Measurement of saturation pH

Saturation pHs of the amlodipine nicotinate and S-(-)-amlodipine nicotinate prepared in 5(1) and 5(2) were measured. In addition, a saturation pH of S-(-)-amlodipine nicotinate in a dihydrate form prepared in accordance with Example 1 of the present invention was measured under the same condition. The results of saturation pH measurement are shown in Table 4.

Table 4.

	Saturation pH
amlodipine nicotinate prepared in 5(1)	6.75
S-(-)-amlodipine nicotinate prepared in 5(2)	6.84
S-(-)-amlodipine nicotinate in a dihydrate form prepared in accordance with Example 1	7.02

As shown in Table 4, S-(-)-amlodipine nicotinate in a dihydrate form prepared in accordance with Example 1 of the present invention has the nearest pH to that of blood (pH 7.4).

10. All statements made herein of my own knowledge are true, and all statements made on information and belief are believed to be true. All statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

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Munchoun Ha

2006, 12, 26,
Date

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